THE CULTIVATION OF *ENDAMOEBA RANARUM*[*]

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When the present authors began the work on the cultivation of the intestinal amoebae in the cold-blooded vertebrates in 1922, it was hoped that the work would not only be of interest in itself, but might throw some light on the cultivation of the forms from man. At that time the only apparent successful cultivation of a species of *Endamoeba* was the work of Cutler, 1918, on *E. histolytica*, and his work was not accepted by many protozoologists. We succeeded first in cultivating an *Endamoeba* from the turtle (Barret and Smith, 1924). In a footnote to the same paper we noted that we had carried one strain of *E. ranarum* for more than two months. We considered the cultivation of *E. ranarum* to be of especial interest because of the great similarity in structure between this form and *E. histolytica* of man. Since our work began, Boeck and Drbohlav (1925) have undoubtedly succeeded in cultivating the parasite of amoebic dysentery, and Chiang (1925) has cultivated a very similar form (*E. histolytica* var. *murina*) from the rat. Drbohlav (1925, a, b, and c) also reports the cultivation of *E. gingivalis*, *E. coli* and *E. aulastomi*. The object of the present paper is to give the details of the successful cultivation of *E. ranarum* from tadpoles.

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CULTURE MEDIUM USED

The culture medium used is the same as that described in an earlier paper in the cultivation of *Blastocystis* (Barret, 1921). It consists of one part of inactivated human serum and nine parts of 0·5 per cent. salt solution.

The H-ion concentration of the medium varies from 7·6 to 7·8 in its natural state. Experimentally it was found that the amoebae would grow in media, ranging from pH 5 to pH 10, but that the most favourable range is between 7 and 8.

PROCEDURE

The lower end of the large intestine of a tadpole is removed, placed on a sterile slide and covered with a small quantity of the culture medium. The contents of the intestine are expressed, and after being mixed with the culture medium, are drawn up in a capillary pipette and placed in two or more tubes containing the medium to a depth of about 50 mm. The material is always placed in the bottom of the tubes as growth takes place only in that portion. The tubes are then placed in the icebox and are allowed to remain undisturbed for from ten days to two weeks, and in some instances longer, after which the sediment is examined for amoebae. One of the mistakes made in the initial attempts to culture the amoebae was an examination of the sediment after too short an incubation period, a fact which may account, in part at least, for the low percentage of positive cultures in our earlier work. When a positive culture is found, transplants are made into several tubes every week or two, depending on how rapidly the amoebae grow. In our routine procedure transplants were never made under one week and some strains did better if left undisturbed for two or even three weeks.

Many obstacles were encountered, which added greatly to the labour of the work. At first, the contents of the lower end of the large intestine were only sub-cultured if they were microscopically found positive for amoebae. Experience showed that this procedure was detrimental to the amoebae, because of drying, etc. Instead, the contents of each intestine were treated exactly as if they contained amoebae and microscopical examination was deferred until the appropriate time for sub-culturing. In the second place, infected frogs and tadpoles are extremely scarce. Some observers have

found as high as 5 per cent. of certain species of frogs infected, while others place the figure at 1 per cent. Owing to this, as well as to the fact that tadpoles are handled in large quantities more easily than frogs, the present workers used tadpoles exclusively. Out of five hundred tadpoles examined, we obtained only six positive cultures, or a little over 1 per cent. infection. In the third place, there were many other organisms in nearly every animal examined which, because of their much more rapid growth, inhibited the multiplication of the amoebae. *Blastocystis* and intestinal flagellates of different kinds were the most troublesome contaminants. In fact, if a culture was contaminated with *Blastocystis* it could neither be freed from them nor successfully transplanted many times thereafter. On the other hand, one of us (Smith) has met with a certain amount of success in freeing cultures of flagellates by subjecting them to varying dilutions of mercurochrome for different lengths of time. The most favourable dilution of mercurochrome was 1-2000 ; the most favourable time of exposure was one hour.

The writers have been able to carry three strains of *E. ranarum*, obtained from tadpoles 220, 222, and 226 respectively, continuously, through numerous sub-cultures, for more than eight months. Furthermore, these strains at their last transfer showed no evidences of dying, and hence, can apparently be kept going indefinitely.

Cysts were present in practically all cultures after a week's growth, their numbers probably depending on the age of the culture. There seems to be, however, no tendency for the active amoebae to disappear entirely in the very old cultures and their places to be taken by cysts. When a culture died, the cysts and amoebae soon disappeared. In this respect, cultures of *E. ranarum* differ from cultures of free-living amoebae, for in the latter, cultures are viable for months owing to the presence of the cysts alone. As stated above, we have been able to obtain cultures that are free of other protozoa ; but all cultures are contaminated with the associated bacteria. From findings in some of the cultures, we believe there is more than one species of amoebae living in the frog. We hope to take up this question later.

A description of the morphology of the amoebae in our cultures is given in the paper by Taliaferro and Fisher, immediately following this one.

CONCLUSION

E. ranarum has been successfully cultivated on a simple medium, and strains have been carried through successive transplants over a period of more than eight months.

LITERATURE CITED

The literature cited in the present paper is given at the end of the paper by Taliaferro and Fisher, which follows.